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TREATMENT

(57) Abstract

There is described a method for the production of surface-coagulated pet food and feed for farmed fish, fur-bearing animals, birds and other animals, which is characterised in that the paste is made of by-products from the meat and/or fish industry and/or whole fish, plus some energy-giving meal, is heat treated at 100-200 °C for 5-60 seconds to apply to the feed a surface film of coagulated protein, the total heat supplied (time x temperature) being controllable when the buoyancy is adjusted in fish feed, whereupon the feed is cooled quickly after coagulation.

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5 PRODUCTION OF PET FOOD AND WET FODDER FOR FUR-BEARING ANIMALS
AND FISH BY SURFACE TREATMENT

10 Surface coagulation of ground meat and/or fish paste is a method which has been
developed for the production of pet food and wet feed for fish and/or fur-bearing
animals. The feed is produced from a paste made of meat and/or fish by-products
and/or whole fish, plus some energy-giving meal. Pellets of ground fish paste are
surface treated with sufficient heat to apply a film of coagulated protein to the feed
particles, whilst the protein in the rest of the feed particles will have a decreasing degree
of coagulation towards the particle core. The method which is described in this
15 application comprises cooking in hot animal and/or vegetable oil. The buoyancy of feed
intended for fish can be adjusted by controlling the amount of heat applied. Cooking in
hot oil (animal and/or vegetable) at different temperatures and for different residence
times provides in addition the possibility of controlling the water content, fat content
and fatty acid content of the feed.

20 By-products from fisheries, animal husbandry and marine aquaculture are a source of
protein and fat, and the amount of by-products from the fish processing and foodstuffs
industry is vast. To use large amounts of fish by-products for our traditional domestic
animals is practically impossible, as such amounts would give the products an off taste.
25 However, this is not a problem if the waste is used for pet food and feed for fur-bearing
animals and/or farmed fish. There is already a limited use of fish waste in dry feed
(feed having < 10% water content) for farmed fish and domestic animals. The
production of dry feed for fish is nevertheless primarily based on the use of fishmeal
and fish oil. The greatest potential for the use of fish waste resides therefore in an
30 extended use of wet feed (feed having > 10% water content) in salmon farming.
Production of feed for fish is used here as an example, but the method described can
also be used for the production of pet food and feed for fur-bearing animals (and
optionally for feed for other animals and birds).

35 When salmon farming started in Norway at the beginning of the 1970s, production was
based on wet feed. The protein and fat contents of this feed varied greatly and its
structure was poor. This led to a high level of feed waste. Dry feed therefore soon

became a great competitor of wet feed. In the course of the last ten to 15 years the use of wet feed for fish has almost come to a halt. The technical quality of dry feed has improved steadily, and intensive research on salmon feed has resulted in a salmon feed which today contains about 40% protein and 30% fat. This has improved feed
5 utilisation, and at the same time has resulted in lower feed production costs as raw protein material is more expensive than fat (oils). Combined with less feed wastage, the consumption of feed has been reduced from about 3.5 kg of dry matter to about 1.1 kg of dry matter per kg of fish produced.

- 10 The cost of salmon production has therefore seen a dramatic reduction since the start in about 1970. However, the cost must in all probability be reduced further, and since feed costs account for more than 40% of the production costs, the price of feed can contribute to the reduction. Fish by-products are very inexpensive (0-70 øre per kg) as to get rid of such waste products often means extra costs for the fish processing industry. Wet feed
15 is therefore once more of interest to fish farming.

- New wet feeds must satisfy the requirements that modern fish farming has with respect to the physical structure of feed, and must not stick together, be crushed or dissolve too quickly in water. The object of the method described in this application is to attain such
20 stabilisation by heat treating the surface of wet feed pellets (which chiefly consist of meat and/or fish by-products and some energy-giving meal) in hot oil. Treatment with hot oil will cause the pellet surfaces to coagulate, so that they neither stick to each other and surrounding equipment nor dissolve in water. The degree of coagulation will decrease towards the core of the pellet, and the depth of the coagulation will therefore
25 be controllable.

- The aim is that central units with access to meat and/or fish waste will in the future be able to provide salmon farmers with quality feed which is considerably cheaper than dry feed. The concept will mean great savings for the salmon farmers who are able to make
30 use of it, whilst ensuring an enhanced utilisation of Norwegian fish resources.

Pet food and fur-bearing animal feed of good quality can be produced using the same equipment, optionally in the same factories.

- 35 Today's production of farmed fish is dry feed based, feeding is largely automated and the amount of feed is easy to control. However, the feed is expensive. Several wet feed

concepts are therefore being tested, including gelling by using alginates and coagulation using microwaves.

- In Norway, the production of dry feed for farmed fish is in the hands of five firms: T. Skretting, Felleskjøpet, Ewos, BioMar and Nor Aqua. All these firms supply feed of good physical and nutritious quality. The major disadvantage of dry feed is however its high price due to production using dry and processed raw materials (fishmeal, animal and/or vegetable oil, soya and pure starch products).
- 5
- 10 Gelled wet feed has a good physical structure and can be fed to its recipients automatically. Alginates however are both expensive and indigestible and may also reduce feed utilisation. Through-coagulation of wet feed by means of microwaves is, in terms of energy, very expensive. This is due to (1): microwave heat can only be produced using electricity, and the generation of microwaves has a heat loss at a
- 15 temperature which is poorly suited for recovery. (2): Heating the pellets with microwaves takes place from the inside. The residual energy in a through-heated feed must therefore inevitably be very high compared with a surface-treated feed. Wet feed produced using microwave heat can therefore not provide very much cheaper feed than today's dry feed.
- 20
- In NO 177731 a method is described wherein the feed is first extruded. To make extrusion possible, about 25% water is added which must be removed again after extrusion. In reality this is a drying method and one purpose of the process is to ensure a fat content. Bumping is achieved since the hot bath is at more than 100°C, and
- 25 cooling takes place in a hot bath at about 50-60°C. In the method of the present invention, the purpose of the fat bath is however to achieve surface coagulation. Shape stabilisation or shaping is also to be achieved. The product is not extruded beforehand, but the paste is cut into suitable lengths which are dropped into the hot bath, and which are thus given a stable shape.
- 30
- Surface-coagulated wet feed has special advantages. The method is based on the use of inexpensive, local, wet primary products, mixed with some energy-giving meal. It does not include the addition of indigestible binders.
- 35
- Sustained high temperature at a low moisture level can cause heat damage to the protein and thus reduce both digestibility and growth. In surface coagulation however, the heat treatment takes place at a high temperature (100-200°C), whilst the feed particles are

wet (<50% dry matter), and the period of heat transfer is very short (5-60 seconds). The surface of the feed particles is subjected to heat sufficient to apply a film of coagulated protein to the feed particles, whilst the protein in the rest of the feed particles will have a decreasing degree of coagulation towards the particle core. The method enhances the digestibility of the protein in comparison with raw ground meat and/or fish paste, probably due to a protein structure which is more readily available for enzymatic cleavage, and thus is also more readily available for absorption. A protein structure of this kind may also bring about a "protein mix effect" with different rates of solubility and digestibility of the protein in the different parts of the feed particle. In this case, this may lead to a more even absorption of amino acids from the intestinal tract and better utilisation of the amino acids for deposits in the muscle.

The economics of energy in the production of wet feed pellets are extremely important as the amounts of raw materials that are to be treated are large. In surface coagulation the consumption of energy for the heat coagulation process will be low initially as only the pellet surface is to be heated. Oil baths (animal and/or vegetable oil) can be heated wholly or partly using steam. More than 90% of the energy in a steam circuit can normally be recovered in such processes. The main amount of the energy consumption in the production will therefore be in the form of residual heat in the finished feed. This energy is difficult to recover.

Cooking in animal and/or vegetable oil and subsequent cooling gives solid pellets having a "meatball consistency" which do not stick, even after freezing. Cooking in oil reduces the water content in the feed and adds extra fat to the feed. This allows the possibility of adjusting the fat content in the feed and also of adding desired fatty acids. The higher fat content, probably together with the formation of gas bubbles in the feed particles during heat treatment, gives the oil-cooked feed entirely different buoyancy than raw ground fish paste. This creates the possibility of controlling the buoyancy in oil-cooked fish feed by controlling the temperature (degree of bumping) and residence time (degree of fat penetration) in the oil bath.

The fat content in the feed particles can also be increased by spraying on oil prior to cooling.

In order to obtain a wet feed pellet having the said physical properties, the pellets of ground meat and/or fish paste must be surface treated with heat, so that they are given a

surface film of coagulated protein. The methods described in this application include as stated above: (1): cooking in hot animal and/or vegetable oil (deep-frying).

5 Examples

On cooking in hot oil, the ground fish paste is pelleted directly in the oil bath (animal and/or vegetable oil, 100-200°C), and is lifted out of the bath and passed into a cooling tunnel by means of a conveyor belt having buckets. The residence time in the oil bath (5-60 seconds) is controlled using the speed of the conveyor belt. The oil is heated
10 using steam pipes or electric elements. The cooled feed is then put into suitable packaging, e.g., large sacks or containers.

I. Preliminary tests on digestibility of wet feed for salmon produced by surface coagulation in hot oil.

15

MATERIALS AND METHODS

Production of test feed

20

Mixing of premix

Two premixes were mixed in a food processor, both consisting of 2 kg gelatinised wheat-oat mixture (70% wheat + 30% oats), with 5 g of indicator added to it for determination of digestibility. Five grams of ytterbium oxide (Yb_2O_3) were added to
25 *Premix 1*, whilst five grams of yttrium oxide (Y_2O_3) were added to *Premix 2*. To ensure an even distribution of the indicator in the premixes, each indicator was first mixed with a small amount of the meal mixture. The rest of the meal was added gradually during the course of 15 minutes, and each premix was subsequently kneaded for 20 minutes.

30 *Mixing of ground fish paste*

Three parts whole herring (27 kg) and one part whole cod (9 kg) were ground twice. Half of the fish mass was frozen in blocks of 750 g, whilst the rest was kneaded as *Premix 1* was gradually added. The finished mixture contained 100 g of wheat-oat mixture per kg and was kneaded for a further 20 minutes. The paste was then left in a
35 cool place over night.

Production of oil-cooked feed

Pellets of suitable size were produced with the aid of a food mixer with a sausage attachment and manual cutting of the string of paste. They were subsequently cooked in sunflower oil for 15 seconds at 180°C in a deep fryer, shaken dry, cooled on a wire tray, and frozen singly.

Production of untreated feed

The block of 750 g of ground fish paste was defrosted in a cold-storage room over night, kneaded in a food mixer as 83 g of *Premix 2* was gradually added. The finished mixture contained 100 g of wheat-oat mixture per kg, and was kneaded for a further 20 minutes. The paste was then rolled out into strings and cut up into suitable pellets.

*Digestibility tests**15 Fish and facilities*

The test was carried out using salmon in seawater, in eight tanks (1m x 1m), with 20 fish of 500 g in each tank. Light was on continuously, and the feed was distributed 24 hours a day with the aid of a belt autofeeder.

20 Dredging out of dung

Dung was dredged out for digestibility measurement on day 8, day 15 and day 18. All the samples of dung from each tank were combined. Due to small amounts of dung, the samples from pairs of tanks within each treatment were combined. Digestibility was further determined following the method described by Austreng (1978).

25 Chemical analyses

The determination of dry matter (105°C, 16-18 hours), ash (combustion at 550°C to constant weight), nitrogen (Kjeldahl N), fat (by hydrolysis) and gross energy (Bomb calorimeter) was carried out at AKVAFORSKs (Norwegian Institute of Aquaculture Research) laboratory at Sunndalsøra. The concentration of yttrium and ytterbium was analysed spectrophotometrically at the Agricultural analysis centre in Ås (Agricultural University of Norway).

Statistical analysis

35 The results were analysed using one-way analysis of variance ($n=2$), with a level of significance selected at $P<0.05$ (5% probability of false rejection of the null hypothesis). The results are given as mean \pm standard error of the mean.

RESULTS AND DISCUSSION

Effects of heat treatment on the structure of the feed

- 5 The composition of the ground fish paste and the feeds is shown in Table 1. The finished paste had a viscous and fine consistency, but with only a 10% content of dry raw materials was still too sticky for pellet production. However, cooking in oil and subsequent cooling gave solid pellets having a "meatball consistency", which did not stick even after freezing. The cooking in oil reduced the water content in the feed from
- 10 60 to 51% and added extra fat to the feed.

Table 1. Composition of feed

15	Feed	1	2
		Untreated	Oil-cooked
<i>Formulation, g/kg (prior to cooking in oil)</i>			
	Whole herring	675	675
20	Whole cod	225	225
	Premix 1 ¹	100	
	Premix 2 ²		100
<i>Chemical composition (after cooking in oil)</i>			
25	Dry matter (DM), g/kg	389.5	490.9
	Protein, g/kg DM ³	427.9	384.3
	Fat, g/kg DM	310.2	405.2
	Ash, g/kg DM	51.5	42.1
	Energy, MJ/kg DM	25.2	27.5
30			

¹ 70% gelatinised wheat + 30% gelatinised oats, with 2.5 g/kg Y₂O₃ added.

² 70% gelatinised wheat + 30% gelatinised oats, with 2.5 g/kg Yb₂O₃ added.

³ N x 6.25.

35

The higher fat content, probably in conjunction with the formation of gas bubbles in the feed particles during the heat treatment, gave the feed cooked in oil a buoyancy entirely

different from that of the raw ground fish paste. The ground fish paste sank immediately, whilst the oil-cooked feed could remain floating for about 24 hours. As a part of the test, a preference study was planned to investigate whether the oil cooking had any effect on the palatability of the paste. This was found impossible to carry out since the salmon took the sinking raw ground fish paste relatively quickly, but took longer to become accustomed to the floating, oil-cooked feed. A palatability study would therefore be greatly affected by the fishes' preference for floating or sinking feed. That all fish groups took feed over time indicates, however, that taste was not crucial to the reluctance of the fish to take the oil-cooked feed in the starting phase of the test.

Effects of heat treatment on digestibility

As can be seen from Table 2, cooking in oil had a positive effect on the digestibility of the feed. Of particular interest was the increased protein digestibility. The digestibility of both protein and energy was improved by 7%.

Table 2. Digestibility of the feeds

Feed	1 Untreated	2 Oil-cooked
Protein ¹	84±0.2 ^b	90±0.2 ^a
Fat	96±0.4	97±0.3
Energy	83±1.1 ^b	89±0.2 ^a

^{ab} indicate statistically certain differences

¹ N x 6.25

Sustained high temperature at a low level of moisture can cause the protein heat damage, and so reduce both digestibility and deposits in the muscle (Opstvedt et al., 1984; Pike et al., 1990). The heat treatment in this test was carried out at a high temperature (180°C), but the feed particles were wet (39% DM), and the duration of the heat transfer was short (15 seconds). Probably only the surface of the feed particles has been subjected to sufficient heat potentially to destroy protein. The protein in the rest of the feed particles seemed to have a decreasing degree of coagulation towards the core of

the particle. All together, this may have given a protein structure which is better available for enzymatic cleavage, and is thus also better available to the fish.

5 Such protein structure may also bring about a "protein mix effect", with different rates of solubility and digestibility of the protein in the different parts of the feed particle. In this case, this may result in a more even absorption of amino acids from the intestinal tract, and better utilisation of the amino acids for deposits in the muscle. However, to examine such effects would call for further tests.

10 The fat digestibility was high in the case of both feeds, and was virtually unaffected by the heat and the extra addition of fat by cooking in oil. However, the extra fat supply reduced the ratio of digestible protein to digestible energy (g digestible protein/digestible MJ) from 17.2 to 14.1 g/MJ. The optimal ratio of digestible protein to digestible energy in salmon feed in the seawater phase is between 16 and 20 g/MJ
15 (Einen and Roem, 1996). When cooking in oil, the paste should therefore be mixed with more protein than fat than in this test, either by using leaner raw fish material or by adding extra fishmeal. If one chooses to add fishmeal, one will at the same time obtain a drier and better consistency of the paste which is to be pelleted.

20 *Conclusions*

This test has shown that cooking ground fish paste in oil results in:

- A stable, coagulated particle structure
- Less water and more fat in the feed particles (more dry matter)
- 25 • Better digestibility of the protein in the feed

Consequently, cooking in oil is a promising form of production for surface coagulated wet feed for salmon with respect to feed utilisation.

Proposed composition

Alternative 1: Use of fatty fish only

5	Raw material, g/kg	Part	Dry matter	Protein	Fat	Carbohydrate
	Herring, 15% fat	850	314	153	128	
	Wheatmeal					
	(gelatinised)	100	92	12	3	70
10	Fishmeal	50	46	35	5	
	Total		452	200	136	70

Digestible energy,

15	MJ/kg	Total	From:	Protein	Fat	Carbohydrate
		9.8		4.27	4.84	0.72
	Percentage of digestible energy			43%	49%	7%

20

Alternative 2: Use of lean fish only

	Raw material, g/kg	Part	Dry matter	Protein	Fat	Carbohydrate
25	Cod, 1.5% fat	750	365	135	15	
	Fish oil	100			100	
	Wheatmeal					
	(gelatinised)	100	92	12	3	70
	Fishmeal	50	46	35	5	
30	Total		403	182	123	70

Digestible energy,

35	MJ/kg	Total	From:	Protein	Fat	Carbohydrate
		9.0		3.88	4.37	0.72
	Percentage of digestible energy			43%	49%	8%

The calculations of digestible energy have been made given the following premises:

5	Protein:	90% digestible
	Fat:	90% digestible
	Carbohydrate:	60% digestible

Both the compositions are well-balanced with respect to the fishes' need for protein, fat and carbohydrates.

10

The compositions include the extreme situations where only fatty fish or lean fish are used. However, the content of wheatmeal and fishmeal are kept constant. Therefore by interpolation one may easily arrive at the correct ratio of raw fish material to oil in new recipes which include both fatty and lean fish.

15

It will necessary to add vitamins, macrominerals and astaxanthin (colouring) to the feed.

II. Test carried out on digestibility of surface coagulated wet feed for salmon after different frying times in oil at 180°C.

20

MATERIALS AND METHODS

Production of test feed

25

The grinding of raw fish material, mixing the paste and deep frying the pellets was carried out by the Norwegian company Alamar AS, Svolvær.

Mixing the ground fish paste

30

The formulation of the ground fish paste is presented in Table 1. The paste was produced in two batches, but using the same composition: 82.9% raw fish material (frozen whole herring and frozen cod scraps), 11.7% dry meal (LT fishmeal and sieved wheatmeal), 4.9% fish oil and 0.5% water.

35

Before the fishmeal was mixed into the paste, 1g of the indigestible marker yttrium oxide (Y_2O_3) per kg was added to it to give 50 mg of Y_2O_3 per kg of the ready mixed paste. To ensure an even distribution of the marker in the fishmeal, the Y_2O_3 was first

mixed into a small amount of the fishmeal in a food processor. The rest of the fishmeal was then gradually added in a larger kneading machine.

- Butylated hydroxytoluene (BHT) was added to the paste as antioxidant. Crystalline
- 5 BHT was first dissolved in alcohol in the ratio of 60g BHT to one litre of alcohol, and 16 ml of this solution was then added per kg of fish oil before being mixed into the paste (50mg BHT per kg paste).

- When mixing the pastes the defrosted raw fish material was first cut up and mixed in a
- 10 large high-speed chopping machine. Then fishmeal, wheatmeal, oil and water were added in turn and mixed into the fish mass in the high-speed chopping machine. Finally the mixtures were passed through a microcutter with a cutting rate of 3000 - 4000 revs/min. The finished pastes had a temperature of about 30°C.

Table 1. Formulation and chemical composition of the paste

	Paste, 1st production	Paste, 2nd production
5		
<i>Formulation, g/kg</i>		
	585	585
	244	244
	49	49
10	68	68
	49	49
	5	5
<i>Chemical composition</i>		
	422	425
15	421	439
	397	353
	62	66

¹ Supplied by K.J. Ellingsen AS, Skrova, Norway

20 ² Supplied by M. Johansen AS, Stamsund, Norway

³ Norse LT-94 (Nordsildmel, Bergen, Norway), with the addition of 1.0g Y₂O₃ (Sigma Chemical Company, St. Louis, Mo. USA), per kg

⁴ Sieved wheatmeal (Statkorn, Oslo Norway)

25 ⁵ Red fish oil (BioMar AS, Myre, Norway), with the addition of 1.0g BHT (Sigma Chemical Company, St. Louis, Mo. USA) per kg

⁶ N x 6.25

30 *Production of deep-fried feed*

The test feed was produced in a modified fish ball cooker, where the mould diameter in the fish ball cutter was 11 mm. The cooker was six metres long and was filled with 1200 litres of oil, which was heated by 28 electric elements of 3.0 - 3.2 kW (a total of 86 - 90 kW). In the cooker there was mounted an inclined conveyor belt which emerged
35 from the oil bath after three metres. By adjusting the speed of this conveyor belt the residence time of the paste balls in the oil bath could be controlled. From the deep-fryer

the ready fried feed moved on a seven-metre open conveyor belt before passing onto a six-metre conveyor belt through an air-cooled tunnel.

Three different feeds (Table 2) were produced from each paste mixture:

5

Feed 1, fried for 15 seconds

Feed 2, fried for 25 seconds

Feed 3, fried for 35 seconds

- 10 Consequently, each feed was produced from two separate paste mixtures in two separate frying batches. The production of each test feed started when the temperature in the oil bath reached 184°C. In each production the temperature then fell gradually to about 179°C. The core temperature in the feed particles was measured in 55 ± 7.9 (mean \pm standard deviation) particles from each production using an electronic thermometer at
- 15 the end of the conveyor belt in the deep fryer, i.e., three metres after the feed had exited the oil bath.

Table 2. Chemical composition of oil-cooked feed

20		1st production			2nd production		
	Seconds in oil bath	15	25	35	15	25	35
	<i>Chemical composition</i>						
	Dry matter(DM), g/kg	502	507	511	497	523	536
	Protein, g/kg DM ¹	354	362	372	364	356	357
25	Fat, g/kg DM	461	492	471	497	517	505
	Starch, g/kg DM	48	49	49	49	44	58
	Ash, g/kg DM	65	57	52	56	49	56
	Energy, MJ/kg DM	28.3	28.4	28.5	28.4	29.1	28.5

30 ¹ N x 6.25

The finished feed was collected in plastic trays, cooled in a cold-storage room (2°C) and frozen in a freezing room (-30°C) on the same afternoon.

35

Digestibility tests

Fish, facilities and dredging out of dung

- The test was carried out using salmon in seawater in 12 test pens (3x7x5 m) with 40 fish of 2.5 kg in each pen. Fish were put out 11 days prior to the start of the test to allow them to become accustomed to the test pens. In the test period the fish were fed according to appetite twice a day.

- Samples of dung inside the pens were dredged out for digestibility measurement on day 19. Digestibility was further determined following the method described by Austreng (1978) using yttrium oxide as an indigestible marker (Austreng et al., 1996).

Chemical analyses

- The determination of dry matter (105°C, 16-18 hours), ash (combustion at 550°C to constant weight), nitrogen (Semi-micro-Kjeldahl, Kjeltex-Auto System, Tecator, Höganäs, Sweden), fat (petroleum ether extraction in a Fostec (Tecator) analyser after HCl hydrolysis, Stoldt (1952)), starch (as described by Storebakken et al. (1991)) and gross energy (Parr 1271 Bomb calorimeter, Parr, Moline, IL, USA) was carried out at AKVAFORSK's laboratories. Yttrium in feed and dung was measured in ash (dissolved in aqua regia (HCl:HNO₃ 2:1 (v/v)) at AKVAFORSK) using an ICP spectrometer (Model 1100, Thermo Jarrel/Ash, Franklin, MA, USA) at the Agricultural analysis centre in Ås.

Statistical analysis

- The results were analysed using one-way (core temperature in feed particles, n=2; digestibility, n=4) and two-way (digestibility, feed * cooking batch) analysis of variance by using the General Linear Models (GLM) procedure in the SAS software program (SAS 1985). For core temperature in feed particles the level of significance was selected as P<0.10 (10% probability of false rejection of the null hypothesis). For digestibility the level of significance was selected as P<0.05 (5% probability of false rejection of the null hypothesis). Significant differences were ranked using Duncan's Multiple Range Test. The results are given as mean±standard error of the mean.

RESULTS AND DISCUSSION

Physical and chemical changes in feed.

- 5 The measurement of the core temperature in the feed particles (Table 3) was somewhat unreliable as the speed of the conveyor belt exiting the oil bath was high. It was therefore difficult to position the measuring probe exactly in the centre of the feed particles. At the same time the cooling of the feed particles was already in progress, as the measurements were taken three metres after the feed particles had emerged from the oil bath. Together, this resulted in a relatively large variation between individual measurements.

15 *Table 3. Core temperature in deep-fried feed particles after different periods in oil bath (Mean±standard error, n=2)*

Seconds in oil bath	15	25	35
20 Core temperature(°C)	86.5±4.9 ^b	95.4±2.8 ^a	96.0±1.1 ^a

Footnotes ^{ab} indicate significant differences within column at 10% level.

- 25 The core temperature in the feed particles which were fried for 15 seconds was significantly lower than the core temperature in the particles which were fried for longer. There was no difference in temperature between the particles which were cooked for 25 seconds and those cooked for 35 seconds. This shows that the core temperature in the feed particles had reached boiling point after 25 seconds frying time at 180°C. That the temperatures measured at 25 and 35 seconds frying time are somewhat lower than 100°C is probably due to the fact that the temperatures were measured very quickly, that the feed particles had already cooled a little, and that the size of the feed particles was uneven, where the core temperature was still lower in the very largest particles. This last-mentioned is also illustrated by the fact that the variation in core temperature decreased when the frying time of the feed particles was increased from 25 to 35 seconds.

The deep frying resulted in an increase in dry matter content in the feed and an increase in the fat content in the feed dry matter (see Table 4). In each production batch the water loss in the feed particles increased as the frying time increased. However, fat absorption was highest after the middlemost frying time, i.e., after 25 seconds. These calculations have been carried out on the basis of analyses of a start sample of the paste from before each production batch, and would consequently be affected by non-homogeneous paste. Differences in particle sizes between the samples for analysis of the different feeds will also affect the calculations, as larger particles have a smaller surface area for fat penetration during deep frying (Orthoefer et al., 1996), and for absorption of fat on cooling (Banks, 1996). It is nevertheless probable that the reduction in fat content from 25 seconds frying time to 35 seconds frying time was an effect of the steam pressure in the frying feed particles reversing some of the oil absorption (Banks, 1996). This is supported by the fact that the fat content in the feed particles increased whilst the core temperature rose, whereas it fell somewhat after the core temperature had stabilised at boiling point.

Table 4. Changes in composition of feed on cooking in oil (Mean \pm standard deviation, n=2)

Seconds in oil bath	Increase in dry matter(%)	Increase in fat (% in dry matter)	Water loss (g/kg paste) ¹	Fat absorption (g/kg paste) ¹
15	7.6 \pm 0.6	10.4 \pm 5.7	163.5 \pm 6.0	74.5 \pm 29.0
25	9.2 \pm 0.9	13.0 \pm 6.0	205.0 \pm 33.9	86.5 \pm 29.0
35	10.0 \pm 1.6	11.3 \pm 5.5	213.5 \pm 41.7	77.5 \pm 37.5

² In relation to the concentration of yttrium in paste and feed.

Open deep frying gives both thermolytic and oxidative degradation of the frying oil, so that the oil will have an increasing content of virtually unavailable and/or toxic products the longer it is used (Márquez-Ruiz and Dobarganes, 1996; Orthoefer and Cooper, 1996; Orthoefer et al., 1996). Tests with rats have shown that mixing in more than 15% of used frying oil in the feed can result in weight reduction, and can in some cases also be

toxic (Márquez and Dobarganes, 1996). The absorption of frying oil in the deep-fried wet feed is well below 15%. Nevertheless it will be important to ensure that the frying oil is changed adequately with a view to both turnover (i.e., the time it takes before all the oil has been changed on refilling) and change of oil in the deep fryer.

5

Digestibility

There was no difference in digestibility of nutrients between feed fried from 15 to 35 seconds, and nor was there any effect of production batch (Table 5). Compared with normal digestibility values of extruded fish feed found by using indigestible markers and dredging out dung (Refstie et al., 1996a, Refstie et al., 1996b; Storebakken et al., 1996), the values in this test were high. Deep frying of wet feed is therefore found to be a gentle heat treatment process, where the residence time of up to 35 seconds in oil at 180°C causes the protein in the fresh raw material very little heat damage, if any.

10

different DP/DE ratios (Table 5). Since the digestibilities of both fat and energy were the same for all the feeds, this was only an effect of different absorption of deep-frying oil at the different frying times (Table 4).

- 5 For dry feed the optimal DP/DE ratio in feed for salmon in the seawater phase is between 16 and 20 g/MJ (Einem and Roem, 1996). Higher water content and possibly better protein quality may perhaps cause the right DP/DE ratio to be lower in wet feed than in dry feed. In the wet feeds in this test the DP/DE ratio was however as low as 12.7±0.3 g/MJ (mean±standard deviation). When deep frying wet feed the paste should
10 therefore contain more lean raw fish material and/or protein meal (fishmeal, soyameal, etc.) If more protein meal is added, the dry matter content in the finished feed will increase simultaneously.

Conclusions

- 15 Deep frying of wet feed pellets (diameter =11 mm) at 180°C for 15, 25 or 35 seconds gives the following results:
- The wet feed pellets are heated through only after 25 seconds frying time.
 - The dry matter content in the wet feed increases as frying time increases, due to
20 vaporisation.
 - The absorption of frying oil increases when the frying time increases from 15 to 25 seconds, but the absorption may be reversed somewhat when the frying time is further increased to 35 seconds.
 - 25 • The digestibility of nutrients is unaffected by frying times within the range of 15 to 35 seconds.

We therefore conclude that deep frying of wet feed is a gentle heat treatment process, where frying in oil at 180°C for up to 35 seconds does not cause the protein in the feed
30 any heat damage.

III. Tests on digestibility of astaxanthin in surface coagulated wet feed for salmon after
35 different frying times in oil at 180°C.

Table 5. Digestibility of oil cooked feed and the relationship between digestible protein and digestible energy in the feed after different periods in oil bath (Mean±standard error, n=4)

<u>One-way analysis of variance in feed</u>					
Seconds in oil bath	Digestibility (%)				DP/DE ¹
	Dry matter	Protein	Fat	Energy	(g/MJ)
15	94.8±0.2	89.0±0.1	93.8±0.5	88.8±0.4	12.7±0.1
25	94.9±0.2	88.9±0.2	93.2±0.7	88.9±0.4	12.5±0.1
35	95.1±0.1	89.3±0.3	93.4±0.7	88.9±0.3	12.8±0.1

Two-way analysis of variance with interaction

Effect of

Seconds in oil bath	N.S.	N.S.	N.S.	N.S.	*
Cooking batch	N.S.	N.S.	N.S.	N.S.	N.S.
Seconds x batch	N.S.	N.S.	N.S.	N.S.	*

Footnotes ^{abc} indicate significant differences in the column at the 5% level

* indicates significant differences at 5% level

N.S. indicates no significant differences

¹ Grams digestible protein per digestible MJ

Preliminary studies of deep frying of wet feed for 15 seconds at 180°C in fact showed a positive effect on the availability of the feed protein (Refstie and Austreng, 1996). When compared, the results from this and the preliminary test may suggest that deep frying for up to 35 seconds has the same positive effect on feed protein.

As regards the ratio of digestible protein to digestible energy (DP/DE, g/MJ) in the various feeds, the differences were small. Nevertheless, different frying times gave

MATERIALS AND METHODS

Production of test feed

5

Mixing of ground fish paste

The formulation of the ground fish paste is presented in Table 1. The paste was produced in two batches, but following the same recipe: 82.9% raw fish material (frozen whole herring and frozen cod scraps), 11.7% dry meal (LT fishmeal and sieved wheatmeal), 4.9% fish oil and 0.5% water.

10

Carophyll Pink, corresponding to 30 mg/kg wet weight, was added to the two different productions of paste. In the case of the first production of paste Carophyll Pink was dispersed in hot water (50°C) by stirring for about 30 minutes and then stirred into the oil phase. In the case of the second production of paste dry Carophyll Pink was added together with yttrium oxide (Y_2O_3). Before the fishmeal was mixed into the paste 1 g of the indigestible marker Y_2O_3 per kg was added to it to give 50 mg Y_2O_3 per kg of the ready mixed paste.

15

To ensure an even distribution of the marker in the fishmeal, Y_2O_3 was first mixed with a small amount of the fishmeal mixture in a food processor. The rest of the fishmeal was then gradually added in a larger kneading machine.

20

Butylated hydroxytoluene (BHT; 2,6-di-*tert*-butyl-p-cresol) was added to the paste as an antioxidant. Crystalline BHT was dissolved in ethanol (60g BHT/l). The solution (16 ml) was added to the fish oil before being mixed into the paste (50 mg per kg paste).

25

When mixing the pastes, the thawed raw fish material was first cut up and mixed in a high-speed chopper (Schneidmischer 325, Krämer-Grebe, Wallau/Lahn, Germany). Then fishmeal, wheatmeal, oil and water were added in turn and mixed into the fish mass.

30

The mixtures were then comminuted by means of a microcutter with a cutting speed of 3000 to 4000 revs/min. The finished pastes had a temperature of about 30°C.

35

Table 1. Formulation and chemical composition of the paste

	Paste 1st production	Paste 2nd production
5		
<i>Formulation, g/kg</i>		
	585	585
	244	244
10	49	49
	68	68
	49	49
	5	5
<i>Formulation, mg/kg</i>		
15	30	30
<i>Chemical composition, analysed</i>		
	422	425
	421	439
	397	353
20	120	142
	62	66

¹ Supplied by K.J. Ellingsen AS, Skrova, Norway

² Supplied by M. Johansen AS, Stamsund, Norway

25 ³ Norse LT-94 (Nordsildmel, Bergen, Norway) with an addition of 1.0g Y₂O₃ (Sigma Chemical Company, St Louis, Mo, USA) per kg

⁴ Sieved wheatmeal (Statkorn, Oslo, Norway)

⁵ Red fish oil (BioMar AS, Myre, Norway) with the addition of 1g BHT (Sigma Chemical Company, St. Louis, Mo. USA) per kg

30 ⁶ N x 6.25

Production of deep-fried feed

35 The test feed was produced in a modified fish ball cooker, where the mould diameter in the ball cutter was 11 mm. The cooker was six metres long, and was filled with 12 litres of oil, which was heated by 28 electric elements of 3.0 - 3.2 kW (in total 86-90 kW). In the cooker there was mounted an inclined conveyor belt which emerged from the oil

bath after three metres. By adjusting the speed of the conveyor belt the residence time of the paste balls in the oil bath was controlled. The ready-fried feed was conveyed further on an open conveyor belt (seven metres) and then through an air-cooled tunnel (six metres).

5

Three different feeds (Table 2) were produced from each paste mixture:

Feeds 1 and 4 cooked for 15 seconds

Feeds 2 and 5 cooked for 25 seconds

10

Feeds 3 and 6 cooked for 35 seconds

Each feed was consequently produced from two separate paste mixtures in two separate frying batches. The production of each test feed started when the temperature in the oil bath reached 184°C. The temperature then fell gradually to 179°C. The core

15 temperature in the feed particles was measured in 55 ± 7.9 (mean \pm standard deviation) particles for each production using an electronic thermometer at the end of the conveyor belt in the deep fryer, i.e., three metres after the feed had exited from the oil bath.

20 *Table 2. Chemical composition of oil-cooked feed*

Seconds in oil bath	<u>1st production</u>			<u>2nd production</u>		
	15	25	35	15	25	35
25 <i>Chemical composition</i>						
Dry matter (DM), g/kg	502	507	511	497	523	536
Protein, g/kg DM ¹	354	362	372	364	356	357
Fat, g/kg DM	461	492	471	497	517	505
Starch, g/kg DM	48	49	49	49	44	58
30 Ash, g/kg DM	65	57	52	56	49	56
Energy, MJ/kg DM	28.5	28.4	28.5	28.4	29.1	28.5

¹ N x 6.25

35

The finished feed was collected in plastic trays, cooled in a cold-storage room (2°C) and frozen in a freezing room (-30°C) the same afternoon.

Digestibility tests

5 *Fish, facilities and dredging out of dung*

The test was carried out using salmon in seawater, in 12 test pens (3x7x5 m), with 40 fish of 2.5 kg in each pen. The fish were set out 11 days prior to the start of the test to allow them to become accustomed to the test pens. In the test period the fish were fed according to appetite twice a day.

10

Samples of dung inside the pens were dredged out for digestibility tests on day 19.

Chemical analyses

The determination of dry matter (105°C, 16-18 hours), ash (combustion at 550°C to
15 constant weight), nitrogen (Semi-micro-Kjeldahl, Kjeltex-Auto System, Tecator, Höganäs, Sweden), fat (petroleum ether extraction in a Fostec (Tecator) analyser after HCl hydrolysis, Stoldt (1952)), starch (as described by Storebakken et al. (1991)) and gross energy (Parr 1271 Bomb calorimeter, Parr, Moline, IL, USA) was carried out at AKVAFORSK's laboratories. Yttrium in feed and dung was measured in ash
20 (dissolved in aqua regia (HCl:HNO₃ 2:1 (v/v)) at AKVAFORSK) using an ICP spectrometer (Model 1100, Thermo Jarrel/Ash, Franklin, MA, USA) at the Agricultural analysis centre in Ås.

Analysis of astaxanthin in dung and feed.

25 The dung was frozen on dry ice immediately after being dredged out in order to avoid decomposition of carotenoid. The samples were stored at -80°C until analysis. The sample was defrosted at room temperature and homogenised by stirring with glass rods. About 10.0 g of wet homogenised dung was weighed in and treated enzymatically with protease (Maxatase®P, 30mg; International Biosynthetics, Rijswijk, the Netherlands) in
30 distilled water (10 ml) in an ultrasonic water bath (30 mins, 50°C) in order to break down the gelatine in Carophyll Pink™. Ethanol (100 ml) was added in a volumetric flask and dichloromethane was filled to 250 ml. An aliquot (10 ml) of the extract was purified using column chromatography with silica gel 60 (Merck, Darmstadt, Germany, No. 7733), and the eluate was steamed off. The sample was dissolved in an accurately
35 determined volume (typically 1 ml) of *n*-hexane: acetone (86:14) and filtered (0.45 µm; Minisart SRP15, Sartorius, Germany) directly into a test tube for HPLC and stored at -80°C until HPLC analysis.

HPLC procedure for analysis of astaxanthin

The samples were analysed isocratically using HPLC on a silica gel column (Merck Hibar, LiChrosorb Si 60.5 μ m particle size; inner diameter 4.6 mm, length 125 mm; flow rate 1.5 ml/min; (35 bar); detection wave length 470 nm) with 14% acetone in hexane as mobile phase, as described by Vecchi et al. (1987). The concentration was calculated with the aid of an external standard. The HPCL used was a Shimadzu LC-10AS liquid chromatograph coupled to a Shimadzu SPD-M6A Photodiode array UV-VIS detector and Shimadzu CBM-10A Communications Bus module. The samples were injected by means of a Shimadzu SIL-10 autoinjector. The chromatograms were reintegrated (Class LC10 software, Shimadzu, Japan) for base line correction. The retention time (R_T) for astaxanthin was about 10.0 mins.

15 *Standards*

External standards were used to quantify the carotenoid content in the samples. Standards having a known concentration were made and run each time samples were analysed. About 3 mg of crystalline all-*trans*-astaxanthin (Hoffmann-La Roche Ltd, Switzerland) was weighed in a 100 ml volumetric flask and dissolved in 4.5 ml chloroform. Then 95.5 ml *n*-hexane was added. An aliquot of this astaxanthin solution (10 ml) was pipetted into a second 100 ml volumetric flask, and 90 ml of 4.5% chloroform in *n*-hexane was added. The absorbance of this 10% solution was measured in a glass cuvette in a spectrophotometer (UV-260, Shimadzu, Japan) against a reference cell containing 4.5% chloroform in *n*-hexane.

25

The concentration was calculated according to the following:

$$\text{Concentration} = \frac{10 * \text{Vol} * \text{Abs}}{E_{1\%, 1 \text{ cm}}}$$

30

wherein

Vol = the volume of solvent used to make the standard

Abs = the absorbency when measuring at 470 nm for astaxanthin

35

$E_{1\%, 1 \text{ cm}} = 2100$ for astaxanthin, in hexane at $\lambda_{\text{max}} = 472 \text{ nm}$.

Determination of digestibilities

Apparent digestibility coefficients (ADC) for astaxanthin in the different feeds were determined using an indirect method after sampling dung as described by Austreng (1978). Yttrium oxide was used as an indigestible marker (Austreng et al., 1996).

- 5 ADCs were calculated according to Maynard and Loosli (1969).

Statistical analysis

- The results were analysed by means of analysis of variance using the General Linear Models (GLM) procedure in the SAS software program (SAS, 1985). For digestibility
10 the level of significance was selected at $P < 0.05$ (5% probability for false rejection of the null hypothesis). The results are given as mean \pm standard error of mean.

RESULTS AND DISCUSSION

15

Process stability of pretreated and untreated Carophyll Pink

- In Table 3 the astaxanthin content in feeds having pretreated or untreated astaxanthin is given for the different treatment times in oil. There was a significant difference between the content of astaxanthin based on dry matter content in the feeds ($p < 0.001$).
20 There was also a significant difference ($p < 0.05$) between feeds to which untreated astaxanthin (45.4 ± 1.7) was added and those to which heat-treated astaxanthin (38.2 ± 0.9) was added. This may suggest that there is a greater loss of astaxanthin through the process when Carophyll Pink is dispersed in water before being added to the feed paste. There were no significant effects of the treatment time on the content of astaxanthin
25 based on dry matter content. However, this will be elucidated in more detail in a later report.

Table 3. Content of astaxanthin in oil-cooked feed immediately after production (mg/kg DM)

Seconds in oil bath	15	25	35
<u>Astaxanthin, mg/kg DM</u>			
Pretreated	38.7±1.0 ^{bc}	36.2±1.3 ^c	39.7±1.8 ^{bc}
Untreated	45.4±2.7 ^{ab}	41.3±3.3 ^{bc}	49.3±1.5 ^a

Footnotes ^{abc} indicate significant differences (p<0.05).

Digestibility

Apparent digestibility coefficients for astaxanthin (ADC) for the different feeds are given in Table 4. There were no significant differences in digestibility of astaxanthin between feed fried from 15 to 35 seconds, although there was a tendency for the feed pretreated with astaxanthin to have a different digestibility after treatment for respectively 15 and 35 seconds (Table 4). Compared with digestibility values for canthaxanthin found when using indigestible markers and dredging out dung (Torrissen et al., 1990), the values in this test were very high. Comparable results were obtained for astaxanthin in rainbow trout (Choubert and Storebakken, 1996; Bjerkeng et al., 1997). Deep frying of wet feed is therefore found to be a relatively gentle heat treatment process, where a residence time of up to 35 seconds in oil at 180°C does not cause any significant damage to the fishes' ability to utilise astaxanthin.

Table 4. Digestibility coefficients (ADC) for untreated and water-dispersed astaxanthin in oil-cooked feed treated for respectively 15, 25 and 35 seconds in an oil bath (180°C)

Seconds in oil bath	ADC (%)	
	Heat-treated	Untreated
15	85.5±0.7 ^t	82.2±1.7
25	84.9±1.0	80.1±1.1
35	77.8±4.1 ^t	81.0±1.0

Footnotes ^t indicate trends (0.05 < p ≤ 0.1).

There was a mild tendency for the digestibility of astaxanthin to diminish as the treatment time in the oil bath increased, Table 5.

There were no significant differences in digestibility of astaxanthin as a result of any pretreatment of astaxanthin, Table 6. One would in fact expect a somewhat higher digestibility of astaxanthin in fish which were given pretreated astaxanthin because the concentration of astaxanthin in this feed was lower. Lower carotenoid concentration gives higher digestibility (Torrissen et al., 1990; Smith et al., 1992; Choubert and Storebakken 1996).

Conclusions

Deep frying wet feed pellets (diameter = 11 mm) at about 180°C for 15, 25 or 35 seconds results in:

- The digestibility of untreated astaxanthin was very high, probably as a result of adequate dissolution of gelatine during heat treatment in oil.
- Water dispersion of Carophyll Pink leads to a more rapid degradation of astaxanthin.
- By prolonging the heat treatment time one may expect significant loss and reduced digestibility of astaxanthin.

Table 5. The effect of heat treatment time on digestibility (ADC) of astaxanthin in oil-cooked feed.

Seconds in oil bath	ADC(%)
15	83.9
25	82.5
35	79.4
Effect	p=0.15

Table 6. The effect of pretreating astaxanthin on digestibility (ADC) of astaxanthin in oil-cooked feed.

	ADC (%)	
	Heat-treated	Untreated
	82.3	81.3
		N.S.

N.S. indicates no significant differences

Consequently, the conclusion may be drawn that deep frying of wet feed is a gentle heat treatment process, where frying in oil at 180°C for up to 35 seconds does not result in reduced digestibility of astaxanthin. The heat process per se seems to be sufficient to obtain a satisfactory digestibility of astaxanthin from Carophyll Pink without any apparent need for pretreatment.

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Patent claims

1.

A method for the production of surface-coagulated pet food and feed for farmed fish, fur-bearing animals, birds and other animals, characterised in that the paste is made of by-products from the meat and/or fish industry and/or whole fish, plus some energy-giving meal, is heat-treated at 100-200°C for 5-60 seconds to apply to the feed a surface film of coagulated protein, the total heat supplied (time x temperature) being controllable when buoyancy is adjusted in fish feed, whereupon the feed is quickly cooled after coagulation.

2.

A method for the production of surface-coagulated pet food and feed for farmed fish and fur-bearing animals according to Claim 1, characterised in that the ground fish paste is pelleted, surface coagulated in a hot oil bath (animal and/or vegetable oil) and the water content, fat content and specific fatty acids content of the feed are adjusted by controlling the residence time in the oil bath and/or the temperature in the bath, and subsequently the ground fish paste is lifted out of the oil bath for cooling.

3.

A method for the production of surface-coagulated pet food and feed for farmed fish and fur-bearing animals according to Claim 1, characterised in that surface-coagulated pellets are sprayed with oil (animal and/or vegetable) prior to cooling.

4.

A method for the production of surface-coagulated pet food and feed for farmed fish and fur-bearing animals according to Claims 1 to 3, characterised in that the surface coagulation is carried out to improve the digestibility of a ground meat and/or fish paste.

INTERNATIONAL SEARCH REPORT

1

International application No.
PCT/NO 97/00093

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A23K 1/00, A23K 1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, DIALINDEX (FOODSCIENCE), STN INDEX (AGRICULTURE)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0579095 A2 (NORSK HYDRO TECHNOLOGY B.V.), 19 January 1994 (19.01.94) --	1-4
A	EP 0208013 A1 (HOSOKAWA, TSUYOSHI), 14 January 1987 (14.01.87) --	1
A	FR 2525439 A (IBP, INDUSTRIE BUITONI PERUGINA SPA), 28 October 1983 (28.10.83) -- -----	1

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "B" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

8 July 1997

Date of mailing of the international search report

22-07-1997

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INTERNATIONAL SEARCH REPORT

Information on patent family members

01/07/97

International application No.

PCT/NO 97/00093

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0579095 A2	19/01/94	AU 664906 B AU 4186293 A CA 2100393 A NO 177731 B NO 932386 A	07/12/95 20/01/94 16/01/94 07/08/95 17/01/94
EP 0208013 A1	14/01/87	JP 1013348 B JP 1531267 C JP 60156369 A	06/03/89 15/11/89 16/08/85
FR 2525439 A	28/10/83	NONE	

